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INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

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SECOND QUARTERLY PROGRESS REPORT
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HYPOXIA, HYPOTHERMIA AND RADIATION RESPONSE IN PEROGNATHUS

J. J. Gambino, R. G. Lindberg and P. Hayden

INTRODUCTION

High $LD_{50/30}$ values exhibited by two species of pocket mice, Perognathus longimembris and P. formosus, suggests a natural resistance to whole body irradiation in this genus unparalleled among the mammals (2). Continuing investigations are concerned with the nature of this resistance (3,4,6). These investigations are being conducted at both the cellular and the organismic levels.

Survival or death in irradiated multicellular animals is dependent upon the ability of a critical number of cells in systems vital to the integrity of the organism to survive and to repair injury. Variation in response of cells to irradiation may reflect certain intrinsic characteristics which allow them to be more or less sensitive. For example, radiation lethality in cells has been correlated with nucleic acid content, structure, and ploidy (5).

Variation in radiation sensitivity of cells may also reflect alterations in the internal mileau. The protective effect of low oxygen tension is an example of this kind of control over radiation response. The oxygen-effect may be acting in the pocket mouse to produce the observed low radiation sensitivity in this species (3). In order to test this hypothesis an experiment was designed to determine whether hypoxia of critical radiosensitive tissues is present in the pocket mouse during irradiation, conferring whole body

protection. It was postulated that if hypoxia (i.e., via biochemical or pharmacological mechanisms) is already operating to raise the LD_{50} significantly, the mechanism would be saturated and further tissue hypoxia as produced by a hypoxic atmosphere would not increase survival appreciably.

This report is concerned primarily with the effect of concomitant hypoxia and high level radiation exposure in the pocket mouse. However, because Perognathus has the capability to undergo hypothermia when exposed to adverse situations, it is difficult to produce hypoxia in this species. Techniques had to be devised to produce hypoxia and to judge its severity. The results of these pilot experiments appeared interesting enough to warrant full reporting.

Part I of this report is concerned with techniques of producing hypoxia in pocket mice. Part II is concerned with the response of Perognathus to Co^{60} irradiation while in severe hypoxia.

PART I

HYPOXIA AND HYPOTHERMIA IN THE POCKET MOUSE

In an effort to produce hypoxia in Perognathus longimembris as part of a radiation effects problem, we found that this species responds to decreased available oxygen as do other mammalian hibernators. That is, as ambient oxygen is reduced, the pocket mouse adjusts to the potentially damaging situation by reducing its body temperature. This response is possible only if the oxygen decrease is slow enough, allowing the animal time to cool. Cooling rate must be sufficient to reduce its metabolic need for oxygen to below that which is available. In this way, pocket mice, like most hibernators, exhibit "hypoxic tolerance".

When body temperature reaches ambient, however, continued reduction of ambient oxygen can be as disastrous to the pocket mouse as it is to non-hibernators. That is, tissue and cellular anoxia quickly ensue and death occurs within a very short period.

The purpose of this series of experiments was to find the best method of producing hypoxia in pocket mice, to determine criteria for judging the severity of hypoxia, and to ascertain the maximum duration of hypoxia in this species that is compatible with survival.

Materials and Methods

Adult Perognathus longimembris of both sexes were used. These animals had been in our holding facility for 6 months to 1 year; therefore, they are all assumed to be young adults.

Hypoxic conditions were produced by flowing into an animal chamber a nitrogen-oxygen mixture. The chamber was a tall 1000 ml spoutless beaker fitted with a rubber stopper and bored to take incurrent and excurrent air tubes and temperature leads. Oxygen flow was controlled to obtain the desired oxygen concentration in the mixture. The chamber was small enough and the gas flow rapid enough (400-600 ml/min) to ensure rapid equilibrium when O_2 tension was changed. Outflow oxygen concentrations lagged only slightly behind changing inflow concentrations. A Beckman expanded scale pH meter with an oxygen adaptor for a polarographic oxygen sensor was used to measure inflow and outflow oxygen concentration.

Deep body temperature and ambient temperature were simultaneously recorded in many of the trials. Body temperature was obtained with a thermistor rectal probe. Experiments were conducted at room temperature and at $10^{\circ}C$. A constant temperature water bath was used to maintain the $10^{\circ}C$ temperature.

Protocol for each trial differed slightly. Usually the animal was instrumented and allowed to acclimate in the chamber in air for 1/2 to 1 hour before hypoxic conditions were started. Oxygen was then decreased in increments and visual observations were made as the hypoxic conditions became increasingly severe. Since techniques for measuring in vivo intra- and extracellular oxygen tension in small mammals are extremely difficult, clinical signs and mortality were used to judge the degree and time limits of hypoxia. Ultimately the animals either died from oxygen lack or were returned to normal ambient and survived after longer or shorter periods at reduced oxygen.

Results

Table 1 presents data on individual pocket mice which were subjected to either acute or stepwise reduction of oxygen in a nitrogen-oxygen breathing mixture at either room temperature or at 10°C. At least eleven animals were administered 5.0% oxygen directly from air with no ontoward effects. Individual animals survived for periods ranging between 14 and 140 hours in this oxygen concentration with no apparent stress or hypoxia damage. Low ambient temperature was not required for this prolonged survival.

Acute depression of ambient oxygen to levels below 5.0%, however, was not well tolerated. Death occurred within 23 minutes in one animal (L-1152) administered 4.0% oxygen directly from air. In another (L-1158) death occurred within 6 minutes after reducing the chamber atmosphere from air directly to 3.3% oxygen. Complete anoxia was tolerated less than 10 minutes in one animal (L-1142) which had been made hypothermic by prior 5.0% oxygen treatment (See also Fig. 1, Chart 4).

Of the eleven animals that were placed on 5.0% oxygen, several were kept there for survival studies and others were subjected either gradually or sharply to even lower oxygen partial pressures. Results show that survival is marginal below about a 3.0% oxygen concentration. One animal (L-1146) survived 36 minutes at 2.8% oxygen after a period of 3-1/2 hours at 5%. It was in severe hypoxia stress prior to restoration of air. Another (L-1155) died after 35 minutes in 2.6% oxygen. It, too, was in severe hypoxia stress. Survival in oxygen concentrations below 3.0% is possible in Perognathus but only after the animal is acclimated by a slow stepwise oxygen reduction. Survival time appears to be approximately 20 to 30 minutes.

A series of experiments were carried out on groups of Perognathus to determine how well groups of 10 or more animals administered hypoxia in a

TABLE 1

Response of individual pocket mice to acute or stepwise reduction of oxygen in a nitrogen-oxygen breathing mixture

Animal No. & Sex	Ambient Temp. °C	Step 1 pO_2	Step 1 Duration	Step 2 pO_2	Step 2 Duration	Step 3 pO_2	Step 3 Duration	Reason for Terminating Experiment
L-1155 ♂	22	5.0%	1hr.27m.	3.9%	3hrs.13m.	2.6	35 m.	Died
L-1154 ♂	22	5.0%	21 hrs.	4.8%	1hr. 35m.			Experimenters Choice
L-1156 ♂	22	5.0%	2 hrs.	4.0%	1hr. 40m.	3.2%	40 m.	Experimenters Choice
L-596 ♀	22	5.0%	140 hrs.					Experimenters Choice
L-1152 ♂	22	4.0%	23 m.					Died
L-1143 ♀	10	5.0%	2hrs.40m.	21.0%*	8 m.	5%	18hrs.	Experimenters Choice
L-1151 ♀	10	5.0%	4hrs.30m.	4.0%	30 m.			Experimenters Choice
L-1153 ♀	10	5.0%	14hrs.					Died
L-1157 ♂	10	5.0%	77hrs.					Died (malfunction of heater)
L-1146 ♂	10	5.0%	3hrs.25m.	2.8%	36 m.			Experimenters Choice
L-1158 ♀	10	3.3%	< 6m.					Died
L-1142 ♂	10	5.0%	17hrs.	0%	<10 m.			Died

*Animal appeared dead but was found to be alive - experiment resumed (see Fig. 1, Chart 3).

single chamber tolerate stepwise reduction of oxygen to 2.6%. Table 2 summarizes the response in these groups. During these group exposures animals were severely stressed at the lower oxygen levels. Signs of anoxia included blue coloration (especially well demonstrated in the tail), hyperventilation, gasping, struggling, and finally apnea. Several animals that had stopped breathing long before termination of the experiment and appeared dead when removed from the chamber gasped after several minutes in air. Some of these were subsequently revived by oxygen administration.

The low oxygen concentration (2.6-2.8%) was chosen for these group exposures to be certain that all animals in the chamber would become hypoxic. This oxygen concentration is marginal for survival in this species and every animal in the chamber appeared severely stressed by this procedure. Of 40 animals treated in this manner, 28 survived. All survivors, however, appear active and healthy at the time of this writing (2 months later). The treatment leaves no outwardly visible residual effects.

Deep body temperature was continuously monitored in a number of individual Perognathus exposed to reduced oxygen concentration while in a 10°C ambient chamber. Figures 1 and 2 show the manner in which body temperature falls when available oxygen is reduced. Figure 1 has records of animals that went directly from air to 5.0% oxygen. In all cases, in a 1-2 hour period, body temperature fell to within a degree or two of ambient. (Records shown in figures are thermistor records and show only relative differences between body temperature and ambient. Values must be converted from calibration curves to show actual temperatures.) During the period of temperature drop the animal gradually reduces its activity until it finally becomes torpid. At this time it appears to be in a natural hypometabolic state. When normal oxygen concentration is restored, there is an immediate increase in body temperature and the animal returns to normal in about two hours (Fig. 1, Chart 1).

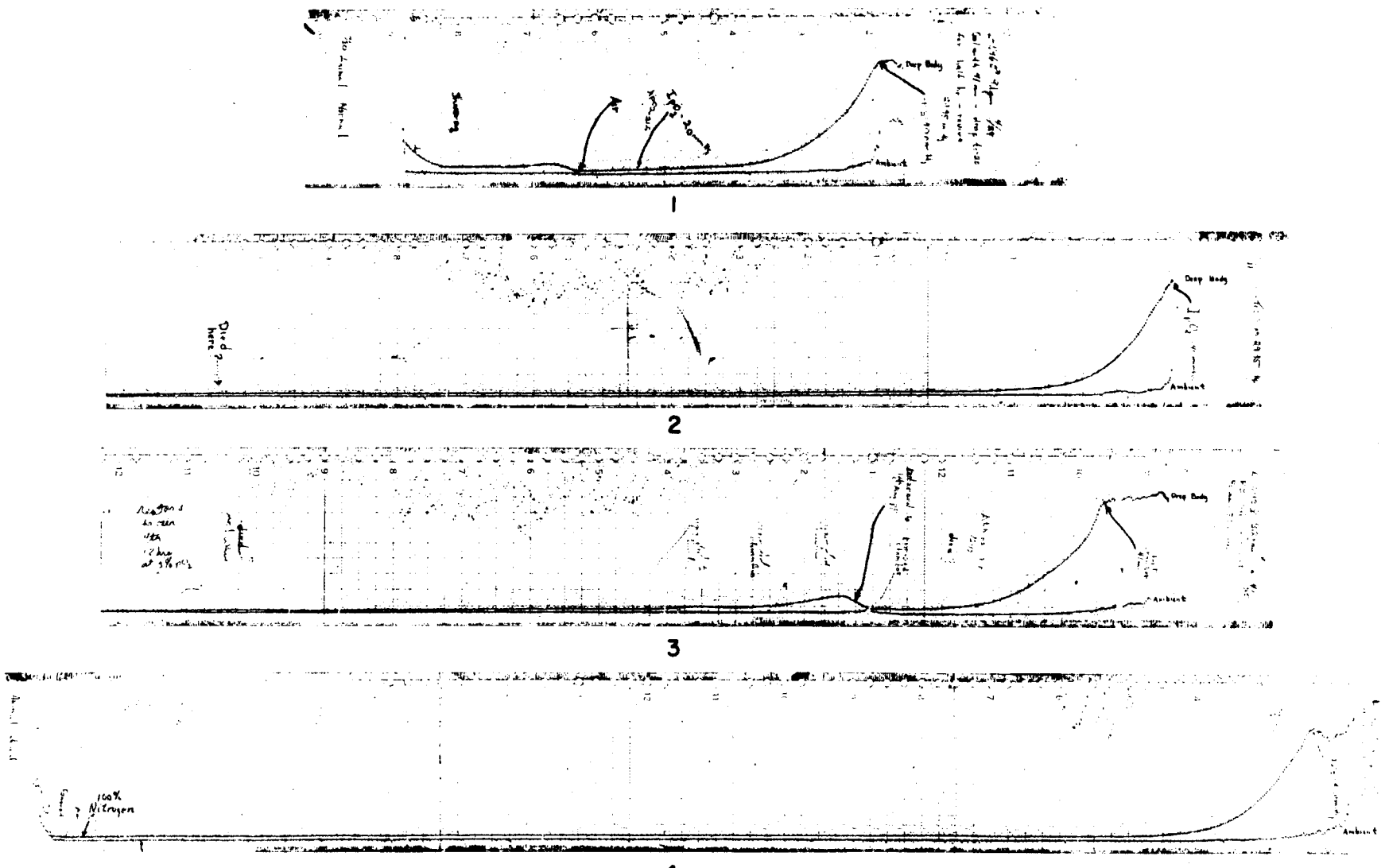
Figure 2 has records of animals that were not placed directly into 5% oxygen, but were reduced more slowly or showed some anomolous response to the

TABLE 2

Response of groups of pocket mice to stepwise reduction of oxygen in a nitrogen-oxygen breathing mixture

No of animals	pO_2 mmHg	$\%O_2$ (Approx.)	Duration (minutes)	Mortality	Comments
10	41	5.3	3	2 dead	T_A = 22.8° - 23.8°C
	160*	21.0	1		
	81	10.5	41		
	61	7.6	33		
	45	5.9	27		
	40	5.3	70		
	33	4.3	67	1 dead	
	20	2.6	20	2 dead	Experiment terminated - air restored, 5 of 10 survived
10	83	10.8	38		T_A = 22.8° - 23.8°C
	63	8.0	37		
	42	5.5	85		
	33	4.3	70	1 dead	
	21.5	2.8	20		Experiment terminated - air restored, 9 of 10 survived
20	100	13.1	30		T_A = 19° - 20°C
	80	10.5	30		
	50	6.5	30		
	40	5.3	30		
	30	4.0	45		
	20	2.6	20	6 dead	Experiment terminated - air restored, 14 of 20 survived

* Several animals appeared severely stressed. Opened chamber for < min., then started oxygen reduction more slowly.



1

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3

4

DEEP BODY TEMPERATURES OF PEROGNATHUS LONGIMEMBRIS ADMINISTERED 5% OXYGEN IN NITROGEN AT AN AMBIENT OF 10°C

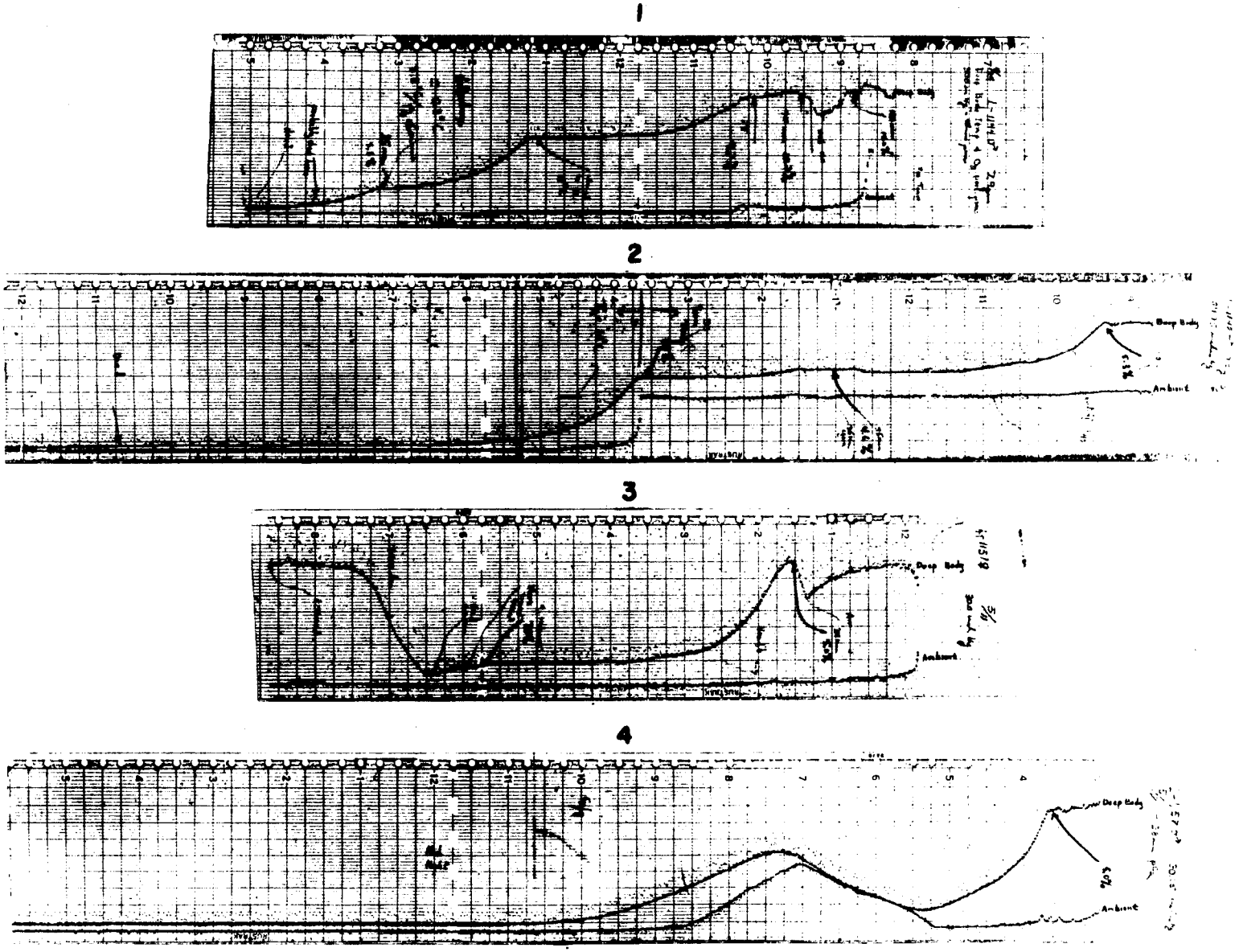
FIGURE 1

low oxygen treatment. In chart 1 the animal was in a 10°C environment and oxygen was reduced gradually from air to 14% down to 3.3%. Body temperature remained normal in an oxygen concentration as low as 12.3%. It dropped off gradually with each succeeding decrease in oxygen concentration with the animal apparently making good adjustment to the 3.3%. For some unknown reason the oxygen had drifted in this trial and at the end of the first hour after starting the 3.3% oxygen mixture, the excurrent concentration read 2.0%. The animal appeared severely stressed and oxygen was returned to about 4.0%. The animal did not recover; however, autopsy revealed the heart still active suggesting that resusitation, had it been applied, may have been successful.

In chart 2 the animal was started at 22°C ambient and administered 5.3% oxygen. Body temperature fell to 26.0°C but remained substantially above ambient (22.3°C). This represents a drop in body temperature of about 10°C. The oxygen was decreased to 3.9% and the chamber plunged into the cold water bath reducing the ambient to 10°C. The animal appeared to make the normal adjustment to the low oxygen concentration at low temperature; however, it died within 7 hours.

Chart 3 shows one animal that was placed in 5.0% oxygen at 10°C ambient, but never dropped its temperature to ambient. After a short period in 3.9% oxygen air was restored and the animal progressed rapidly to normal.

Chart 4 shows an animal that was subjected to two malfunctions of the heating unit in the water bath. The first occurred only a short while after the experiment started and the animal survived. The second occurred after 77 hours, killing the animal (not shown in the record). It is noted in this chart that the animal's body temperature coincides with ambient as the heating occurred but lagged as the chamber returned to low ambient temperature.



DEEP BODY TEMPERATURES OF PEROGNATHUS LONGIMEMBRIS ADMINISTERED VARIOUS CONCENTRATIONS OF OXYGEN IN NITROGEN AT 22°C OR 10°C AMBIENT TEMP

FIGURE 2

Discussion

The ability to survive exposure to lowered environmental oxygen is not the same in all mammals. For most adult mammals, hypoxic environmental conditions quickly result in a chain of events leading to tissue hypoxia, reduced cellular oxygen tension and, if oxygen lack is severe enough, death.

Some mammals, on the other hand, are able to withstand remarkably long periods in extremely low ambient oxygen levels. Many hibernating mammals have this ability, which is based on body cooling and keeping oxygen "demand" below "supply". According to Van't Hoff's law, body cooling decreases metabolic oxygen requirement and thereby prevents or postpones anoxia damage.

This ability, as it is seen in hibernators, appears to be an amplification of a phenomenon common in many other mammals. In Wistar rats, for example, as environmental oxygen is reduced, body cooling occurs and hypoxia survival is enhanced. The ability is not nearly as well developed in the rat as it is in the hibernating ground squirrel (1). At room temperature both rats and ground squirrels survive at least 2 hours in a 5.5% ambient oxygen concentration. During this period body temperature of the rats dropped $7^{\circ}C$ while that of the ground squirrel dropped $9^{\circ}C$. In a 4.1% oxygen atmosphere rats died in 30 minutes, while ground squirrels survived for at least 2 hours. The ground squirrel showed a $10^{\circ}C$ decrease in body temperature in this atmosphere.

Ability to cool may not be the whole story. The hamster is able to survive 4.1% oxygen for 2 hours; with only half the temperature drop exhibited by ground squirrels (1). It has been suggested that in hibernators mechanisms other than passive body cooling are operating. High oxygen affinity of hibernator hemoglobins, unusual vasomotor activity, increased anaerobic metabolism are listed among the adaptations possibly available to the hibernator.

Whatever the mechanism, it is generally agreed that hibernators have high tolerance to hypoxic conditions while non-hibernators do not.

Perognathus is sometimes classed as a hibernator. Indeed, it has the ability to undergo periods of hypometabolism and appears to posses many of the adaptive mechanisms of hibernators. Our data suggests, also, that Perognathus responds to reduced oxygen in a manner that is reminiscent of hibernators. Its return to normal, however, may not be similar. Ground squirrels in a 7°C chamber made hypothermic by hypoxia do not return to normal when normal atmospheric gas concentration is restored but remain in a "hibernation" state (1). They arouse only after chamber temperature is increased to 15°C. It was suggested that these animals may be in a state of "neural" hibernation but not "endocrine" hibernation (1). In contrast, in hypoxia-induced hypothermia in P. longimembris, arousal occurs immediately after normal atmospheric oxygen is restored.

Interestingly enough, because there is difficulty in making in vivo intra-cellular oxygen measurements in small rodents, there is little comparative data on survival of hibernators and non-hibernators after the onset of tissue hypoxia. In Perognathus, if the reduction in oxygen is sudden and severe, survival time is extremely short. This demonstrates that the rate of oxygen reduction is very important if survival is to occur. If the rate at which oxygen levels are reduced exceed the rate at which metabolic needs are reduced, oxygen lack is certainly evident in Perognathus. This suggests that the hibernator may be not at all tolerant to hypoxia at the cellular level. Their "hypoxia tolerance" may be based solely on their efficiency in reducing metabolic need for oxygen.

We know of no other studies in which animals were maintained in low oxygen for the length of time that they were in this study. Survival in 5% oxygen concentration for 140 hours appears to be a remarkable phenomenon. There is good evidence from our studies that Perognathus longimembris may be able to remain in 5% oxygen for even longer periods. The limiting factor may be nutrition.

When the animal is in this low oxygen environment at 10°C ambient temperature, its body temperature remains within a degree or two of ambient. The animal is, of course, incapable of activity in this condition and does not eat. Even though its energy requirement is low, it appears that the animal must ultimately perish from starvation.

The fact the Perognathus does survive for long periods under these conditions suggests a means of even further reducing payload requirements in space experiments. If normal metabolic activity is not a requirement as in, for example, heavy primary cosmic ray effects studies, long term experiments are even more feasible.

PART II

RESPONSE OF THE POCKET MOUSE TO IRRADIATION DURING HYPOXIA

In a continuing effort to elucidate the mechanism of radiation resistance in pocket mice, a group of Perognathus longimembris were irradiated while they were in severe hypoxic hypoxia. Survival of irradiated hypoxic mice was compared to survival of mice administered the same dose while in a normal oxygen environment. Results suggest that in pocket mice, as in other mammals, hypoxia during exposure enhances survival when administered a supralethal dose of gamma irradiation.

Materials and Methods

Sixty adult Perognathus longimembris were segregated from our holding colony and randomly divided into 3 groups of 20 each. One group received 2100 r total body radiation; the second group received 2100 r total body radiation while hypoxic; and the third group received hypoxia only.

The pocket mice were made hypoxic by a stepwise reduction of ambient oxygen in a nitrogen-oxygen breathing mixture piped into a lucite exposure chamber. The degree of hypoxia was judged by clinical signs of stress and by survival studies in control groups. In preliminary studies it was determined that by reducing oxygen in increments, 75% of a normal population of pocket mice would survive 25 to 30 minutes in 2.6% oxygen at room temperature.*

* Details of method in Part I of this report.

This oxygen concentration was judged sufficient to insure complete hypoxia in all of the mice. A radiation dose rate was selected to obtain, within the 25 to 30 minutes time period, delivery of a total dose sufficient to produce 100% acute (< 30-day) deaths in unprotected pocket mice.

Irradiation was delivered from a 5000 curie Co^{60} source at a dose rate of 105 r/min. The total dose (2100 r) was delivered in about 20 minutes. Both ferrous sulphate and phosphate glass dosimeters were used.

The group irradiated under hypoxia was returned to a normal oxygen atmosphere immediately after irradiation. The total period in 2.6% oxygen was less than 25 minutes. The hypoxia control group had identical treatment except for the irradiation. The group irradiated without hypoxia was handled similarly and administered the same dose under the same conditions as the irradiation-hypoxia group except, of course, for the hypoxic atmosphere.

After irradiation all groups were returned to the holding facility and maintained as reported previously (2,6). They were checked twice daily, in the morning and in late afternoon, and observed for signs of radiation sickness and deaths. Dead animals were autopsied to determine gross pathology.

Results

Survival of mice in this experiment is shown in Table 1. At 30 days post-irradiation all of the animals that survived the exposure period were alive in the hypoxia groups and all of the animals irradiated without hypoxia were dead.

Mortality in the group that received 2100 r without hypoxia occurred in the 8th through the 15th day, with a mean survival time of 11 days. The most common autopsy finding in this group was large amounts of bloody fluid in the small intestine. In addition, respiratory infection characterized by large foci of consolidation or hepatization was seen in 7 of the 20 dead mice. Intracranial hemorrhages as described in earlier reports (3,4) were seen in 4 animals.

TABLE 1

Survival of pocket mice receiving 2100 r total body irradiation with and without hypoxia

Group	Initial Number	Treatment	Number Surviving Exposure Period	Number Surviving at 30 days
A	20	2100 r + Hypoxia	8	8
B	20	2100 r	20	0
C	20	0 r + Hypoxia	14	14

All of the animals that survived hypoxia, both irradiated and controls, are alive and appear healthy at the time of this writing (8 weeks post-irradiation). During the period in which the unprotected irradiated mice were dying, one of the irradiated-hypoxia animals appeared lethargic for about 1 day. Aside from this single observation, hypoxia alone or hypoxia plus 2100 r administered to pocket mice did not effect the survivors (as judged by outward signs).

Discussion

Hypoxic hypoxia, as administered in this experiment, enhances survival in Perognathus longimembris exposed to a lethal dose of whole body irradiation. At this point in the experiment all of the animals that were administered 2100 r without hypoxia protection are dead, while all of those surviving exposure to 2100 r plus hypoxia are alive.

It is difficult to explain the inordinately high mortality during exposure in the group receiving irradiation with hypoxia. It is possible that the combined stresses were too severe, or perhaps our preliminary data was too scanty to account for biologic variability. There is also a possibility that occlusion of the air line may have occurred briefly during the acclimitization period. In spite of the loss of statistical data as a result of this high mortality, the fact that all 8 survivors of the hypoxia-radiation

exposure period are still alive while all of the unprotected mice died before the 16th day post-irradiation, indicates that the hypoxia treatment was successful. The high mortality during exposure of hypoxia-radiated animals confirms the severity of the hypoxia treatment.

On this basis, we must reject the hypothesis that the pocket mouse is resistant to high levels of acute irradiation because it somehow invokes a local hypoxia in critical radiosensitive tissues during irradiation. If hypoxia (i.e., via biochemical or pharmacological mechanisms) was already operating, the mechanism would be "saturated", therefore, added hypoxia (i.e., via hypoxic hypoxia) would not increase protection.

These results corroborate results of previous experiments in which splenectomized pocket mice and pocket mice in a high pressure 100% oxygen atmosphere were administered high radiation doses (3). In these experiments evidence was also elicited suggesting that no hypoxia in critical radiosensitive tissues of Perognathus exists during radiation exposure under standard conditions.

The mortality pattern of animals receiving 2100 r without protection was comparable to results reported previously from this laboratory for the pocket mouse (4). The time of death grouped around the 11th and 12th days post-irradiation follows closely the pattern of pocket mice administered doses ranging from 1400 r to 2000 r in earlier work. Also, autopsy findings were similar to those found in earlier work.

It may be significant that no deaths occur during the first week post-irradiation when gastro-intestinal deaths normally occur in other mammals. The pocket mouse appears to be the only mammal that exhibits 100% survival during the first week following total body irradiation at doses greater than 1000 r. Mice receiving > 2000 r in this experiment survived at least through the 7th day post-irradiation. According to Patt and Quastler, large, pleomorphic, functionally abnormal ω-cells form a coherent sheet lining of

the intestinal lumen after the initial loss of normal epithelial cells (7). The loss of α-cells within the normal villus transit time leaves the gut totally denuded leading to "intestinal radiation death I" in 2nd or 3rd day post-irradiation. In some animals ω-cells may persist for several days and death may be due to bacteremia - "intestinal death II" - between 5-7 days in rodents, or replacement of ω-cells with newly formed crypt cells may occur with ultimate survival.

Judging from gross pathology, severe gastro-intestinal damage is present in pocket mice receiving doses greater than 1000 r. It is possible that α-cells do persist for several days in this animal providing protection during the first days following irradiation. Survival in the 5-7 day period may reflect a general resistance to bacteremia common in native rodents. This conforms with the hypothesis of Roderick that general fitness appears to be an indicator of radio-resistance in mice (8). Death occurring in pocket mice after the 8th day may be attributed to hematopoietic failure, but is probably enhanced by profound damage in the gut epithelium and capillary net, allowing massive hemorrhaging into the intestinal lumen. This hypothesis is supported by two facts; extremely low platelet counts at the 10th day post-irradiation (2), and autopsy findings.

If this hypothesis withstands further testing, it may be possible to conclude that at least three unrelated mechanisms are acting to protect pocket mice from high dose irradiation. One, persistant ω-cells, aiding the animal in the 1-3 day period; two, natural resistance to common pathogens, enhancing survival in the 5-7 day period; and finally, a resistance in bone marrow (possibly rapid regeneration capability) allowing survival in dose ranges that normally are lethal due to hematopoietic death (9).

Since the hypoxia hypothesis which could account for simultaneous protection of bone marrow and gastro-intestinal tissues has now been rejected, intensive investigation of these other mechanisms have been initiated.

SUMMARY

Response of pocket mice, Perognathus longimembris, to low ambient oxygen and to combined hypoxia and radiation was investigated. Perognathus responds to decreased ambient oxygen in the same manner as do mammalian hibernators. As oxygen is decreased body cooling occurs, decreasing metabolic need for oxygen, and, if body cooling is rapid enough oxygen depletion is not evident at the tissue level. This mechanism of hypoxia-induced hypothermia allows high tolerance to extremely low ambient oxygen (2.6 to 2.8% for 1/2 hour in Perognathus). Tolerance is judged in terms of clinical signs of hypoxia stress and duration of hypoxic conditions compatible with survival. Perognathus is capable of surviving up to 140 hours in a 5.0% oxygen atmosphere at room temperature. If 5% oxygen is administered at room temperature, body temperature falls approximately 10°C, remaining several degrees above ambient (22.3°C). If 5% oxygen is administered at 10°C ambient, body temperature falls to within a degree of ambient.

There is evidence that Perognathus tolerates hypoxia at the tissue level no better than non-hibernators.

Perognathus exposed to hypoxic conditions while at 10°C ambient temperature behave similarly to other hibernators. Return to normal after hypoxic conditions are removed, however, is spontaneous and rapid in Perognathus, while in at least one other hibernator similarly treated, return to normal occurs only after ambient temperature was raised.

Pocket mice were administered 2100 r total body Co^{60} irradiation while in severe hypoxia. No deaths have occurred in the 8 week period following

the irradiation exposure. Unprotected pocket mice administered 2100 r died in the 8th-15th day. Hypoxia controls show no death in the same period. It is suggested that the natural radioresistance in pocket mice cannot be explained on the basis of local or general hypoxia invoked during irradiation under standard conditions.

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